Pathogen evasion strategies for the major histocompatibility complex class I assembly pathway

Antony N. Antoniou[1] and Simon J. Powis[2]

[1]*Department of Immunology & Molecular Pathology, Division of Infection & Immunity, & Rheumatology, University College London, Windeyer Institute of Medical Science, London, UK, and* [2]*Bute Medical School, University of St Andrews, Fife, UK*

doi:10.1111/j.1365-2567.2008.02804.x
Received 24 September 2007; revised 22 December 2007; accepted 2 January 2008.
Correspondence: Dr A. N. Antoniou, Department of Immunology & Molecular Pathology, Division of Infection & Immunity, & Rheumatology, University College London, Windeyer Institute of Medical Science, 46 Cleveland Street, London W1T 4JF, UK. Email: anthony.antoniou@ucl.ac.uk
Senior author: Dr Antony N. Antoniou

Summary

Major histocompatibility complex (MHC) class I molecules bind and present short antigenic peptides from endogenously or exogenously derived sources to CD8$^+$ cytotoxic T lymphocytes (CTL), with recognition of a foreign peptide normally targeting the cell for lysis. It is generally thought that the high level of MHC polymorphism, which is concentrated mostly within the peptide-binding groove, is driven by the 'evolutionary arms race' against pathogens. Many pathogens have developed novel and intriguing mechanisms for evading the continuous sampling of the intracellular and intercellular environments by MHC molecules, none more so than viruses. The characterization of immunoevasion mechanisms has improved our understanding of MHC biology. This review will highlight our current understanding of the MHC class I biosynthetic pathway and how it has been exploited by pathogens, especially viruses, to potentially evade CTL recognition.

Keywords: biosynthesis; cross-presentation; ER associated degradation (ERAD); major histocompatibility complex class I (MHC class I); viral evasion

Introduction

Most nucleated cells express major histocompatibility complex (MHC) class I molecules, providing them with protection against invading pathogens by allowing the display of cellular contents to the immune system. The MHC class I molecules can sample both the intracellular and extracellular milieus for defective and foreign proteins by presenting peptide fragments to immune effector cells. The MHC class I–peptide complexes are monitored by cells of both the innate and acquired immune systems, namely natural killer (NK) cells and CD8$^+$ cytotoxic T lymphocytes (CTL), respectively.

Microbial pathogens that access the sustainable microenvironments provided by complex vertebrate organisms must overcome the many facets of the immune system designed to eliminate foreign invaders. In turn, the immune system attempts to unmask and eliminate such pathogens. The constant struggle between host and pathogen is thought to drive an 'evolutionary arms race' between the pathogens and their respective hosts' immune systems. One area of immunobiology that has become increasingly well understood is the biosynthetic pathway of MHC class I assembly. This has been achieved, not only through a greater understanding of the biochemical pathway, but by unravelling mechanisms

Abbreviations: β2m, beta-2-microglobulin; BiP, immunoglobulin binding protein; C, cysteine; CTL, cytotoxic T lymphocytes; CYT, cytoplasmic; ER, endoplasmic reticulum; ERAD, ER associated degradation; H, heavy; HCMV, human cytomegalovirus; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; K, lysine; KSHV, Kaposi's sarcoma-associated herpesvirus; MHC, major histocompatibility complex; NK, natural killer; PDI, protein disulphide isomerase; PLC, peptide loading complex; TAP, transporter associated with antigen processing; TGN, trans-Golgi network; TM, transmembrane; TPN, tapasin; Ub, ubiquitin; UL, unique long; US, unique short.

employed by pathogens, particularly viruses, to evade presentation by MHC molecules.

MHC class I assembly – a chaperone-mediated event

MHC class I molecules are composed of a tripartite complex of heavy (H) chain (45 000 MW), β2-microglobulin (β2m) light chain (12 000 MW) and a peptide between 8 and 10 amino acids in length. MHC class I molecules assemble within the oxidizing environment of the endoplasmic reticulum (ER) via a series of chaperone-mediated events which occur in two main phases: (1) an early assembly pathway, governing the appropriate folding of the H chain with β2m and (2) a later stage characterized by the formation of the 'peptide-loading complex' (PLC) and the acquisition of optimal peptides.[1] Once suitable peptide has been bound, MHC class I molecules exit the ER, transit through the Golgi apparatus to the cell surface to present their cargo to $CD8^+$ T cells. Throughout the pathway, MHC class I molecules are closely scrutinized, with misfolded molecules being discarded through a process referred to as ER-associated degradation (ERAD). This extra level of quality control performed by the PLC distinguishes MHC class I molecules from many other proteins that assemble within the ER.

The early folding stages – prepeptide loading complex

MHC class I H chains are composed of three distinct extracellular domains, a transmembrane (TM) domain and a cytoplasmic (CYT) domain. The α1 and α2 domains, form two α-helices and a series of β-pleated sheets, comprising the walls and floor of the peptide binding groove respectively. The α3 domain adopts an immunoglobulin-like fold, forming the predominant non-covalent interactions with β2m. The H chain possesses four conserved cysteine (C) residues, forming two structurally important disulphide bonds within the α2 and α3 domains between C101–C164 and C203–C259 respectively.

Calnexin and the 'folding cage'

Newly synthesized MHC class I H chains associate with the TM-bound chaperone calnexin,[2] via a monoglucosylated ($Man_9GlcNAc_2Glc_1$) sugar moiety chemically linked to the conserved asparagine at position 86 of the H chain,[3–5] with binding regulated by glucose trimming of nascent N-linked oligosaccharides (Fig. 1 step 2).[6] Structural analysis of the calnexin ER luminal domain revealed that the glycan binding site would place the MHC class I H chain within the extended proline-rich domain (P domain), providing a 'folding cage' for the initial stages of assembly.[7] A semipermeabilized cell system, demonstrated that calnexin can partition and stabilize unfolded H chain away from the ERAD machinery,[8] but in the absence of calnexin, assembly proceeds normally.[9] The P domain also recruits the oxidoreductase ERp57 (Fig. 1 step 3), a member of the protein disulphide isomerase (PDI) family of proteins, which reduce, oxidize or isomerize disulphide bonds.[5,10,11] ERp57 possesses two reactive thioredoxin-like CXXC motifs within the N-terminal and C-terminal domains, referred to

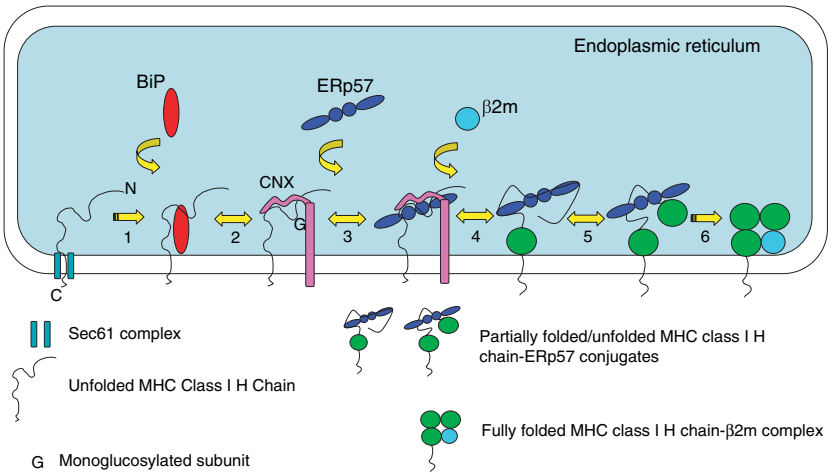

Figure 1. Early stages of major histocompatibility complex (MHC) class I assembly; newly synthesized MHC class I heavy (H) chain is translocated through the Sec61 channel. It remains undetermined whether newly synthesized or misfolding H chain associates with immunoglobulin-binding protein (BiP; step 1). Calnexin (CNX) associates with newly synthesized H chains via a monoglucosylated sugar moiety (G). The relationship between BiP and calnexin-associated H chain remains undefined (step 2). Calnexin can recruit ERp57 (step 3) to aid in the folding of native protein. ERp57 can be found in direct association with MHC class I H chains (steps 4 and 5). MHC class I H chain association with β2-microglobulin (β2m) leads to calnexin displacement and can promote disulphide bond formation (step 6).

as **a** and **a′**, respectively. The spacing between the cysteine residues of the reactive motifs appears to be important in maintaining ERp57 in the appropriate redox active state.[12] ERp57 can form several direct conjugates with the H chain via transient disulphide bonds with C101 and C164, probably reflecting the different folding stages of the H chain (Fig. 1 step 4 and 5).[13,14] The transient interaction between MHC class I H chain and ERp57 could be part of the ERAD pathway for H chains, whereby MHC class I H chains are reduced on route to proteasome degradation.[14,15]

BiP, an undefined association

MHC class I H chains can also be detected in association with the immunoglobulin-binding protein (BiP) (Fig. 1 step 1).[16,17] The precise function of this BiP association is undetermined but BiP has been described to bind transiently or for a prolonged period to newly synthesized or misfolded proteins, respectively.[18,19] It remains unknown whether BiP association is a function of newly synthesized or misfolded MHC class I H chains, though certain alleles can form strong associations with BiP, such as human leucocyte antigen (HLA) B27, which are known to exhibit an enhanced tendency to misfold.[17,20] Alternatively, the association of BiP may depend on the kinetics and stability of certain HLA alleles.[17] Such factors have been described as determining factors for other BiP associating proteins such as components of immunoglobulin.[18]

Pre-PLC subversion-enhanced endoplasmic reticulum associated degradation

It is interesting to note that, to date, no pathogen has targeted chaperones of the early folding pathway to inhibit MHC class I synthesis. This might reflect a requirement for chaperones such as calnexin and ERp57 in the folding of pathogen proteins,[21,22] but it also appears that removing chaperones such as calnexin does not have a dramatic impact on MHC class I expression.[9]

Throughout the folding process, misfolded MHC class I molecules are prone to ERAD,[23,24] a process involving dislocation into the cytosol possibly using the Sec61 complex.[25] Mannose trimming and unfolding of MHC class I H chains can occur before retrotranslocation, a process thought to occur through a putative subcompartment of the ER, termed the 'quality control' compartment.[26,27] The MHC class I H chains are then targeted for proteasome-mediated degradation by the addition of ubiquitin (Ub), a small 76-amino-acid protein, whose C-terminal glycine forms an isopeptide bond with the ε-amino group of lysines (K) or the NH_2 group at the N terminus of proteins.[28] Ubiquitination involves the sequential action of three enzymes, a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2) and a Ub-ligase (E3).[29]

Promoting the degradation pathway

Human cytomegalovirus (HCMV) encodes within its unique short (US) region a series of gene products, expressed during different stages of viral infection, that affect MHC class I biosynthesis and expression. It encodes two gene products US2 and US11,[25] which enhance the degradation of ER-localized MHC class I H chains. US2 and US11 are small TM ER-resident glycoproteins with a small degree of homology to each other[30] that use distinct pathways to achieve the same goal.[31] A cell-free system demonstrated that US2 requires both its TM and CYT domains, while US11 relied only on its TM domain,[31,32] to trigger MHC class I dislocation. US11 depends on MHC class I α1/α2 domain interactions and US2 associates predominantly with the α2/α3 domain,[33] which was confirmed by the structural determination of US2 in association with HLA-A2,[33,34] with further differences in allele sensitivity to US2 degradation attributed to residues within the 180–183 region.[31,35] It was suggested that US2 exhibited a preference for properly folded, β2m-associated MHC class I molecules,[36] but in a β2m-negative cell line, unfolded H chains were susceptible to US2- and US11-mediated ERAD.[37] Once in the cytosol, the H chain is ubiquitinated before deglycosylation[38] by the activity of the cytosolic peptide N-glycanase, Pgn (Fig. 2).[25,39]

MHC class I ERAD; more than one way for waste disposal

US11 and US2 exhibit further differences in their ability to degrade class I molecules depending on the cell type. US11 is more efficient than US2 in MHC class I disposal when expressed in dendritic cells, raising the possibility that pathogens have evolved cell-type-specific mechanisms for immune evasion.[40] The dislocation event is sensitive to changes in the ER redox environment.[15] Degradation mediated by US2, but not that mediated by US11, appears to require the α3 domain C203–C259 disulphide bond,[41] while US11-mediated degradation was compromised in the presence of proteasome inhibitors and in the absence of β2m.[37] The presence of β2m can support disulphide bond formation[42] and alter the chaperone association of H chains,[43,44] indicating that perhaps US11-mediated degradation depends upon certain redox states and/or chaperone activity.

It was apparent that the different criteria for US2- and US11-mediated activity, reflected the use of distinct degradative pathways (Fig. 2). Both US2 and US11 require ubiquitination of the H chain, but employ different components of the Ub-system.[41] This was illustrated by disruption of the E3 Ub-ligase SelL1 affecting US11-mediated degradation, but not US2-mediated degradation.[45] US11 was found to link MHC class I H chains to a 'dislocation complex' comprising Derlin-1 (a human

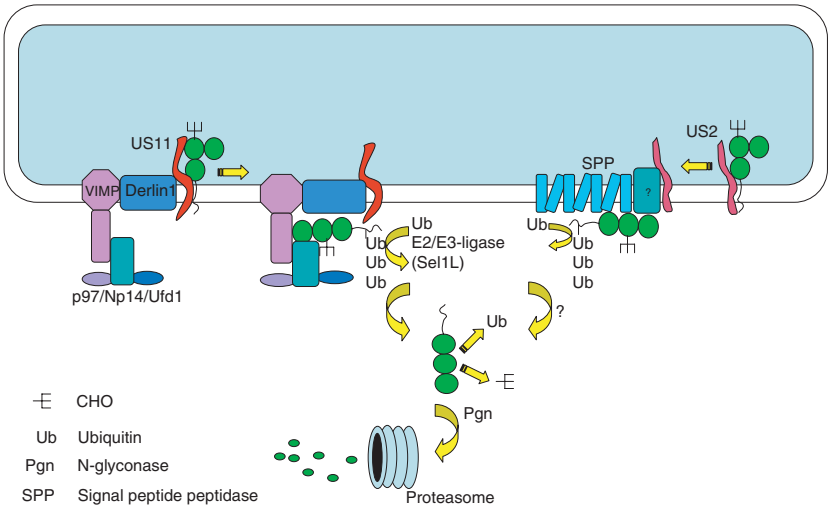

Figure 2. Inhibition of the early stages of assembly; US11 and US2 enhance the endoplasmic reticulum-associated degradation pathway of major histocompatibility complex (MHC) class I heavy (H) chains but through distinct pathways. US11 recruits a dislocation complex composed of Derlin1, VIMP and a p97 complex. The H chain is retrotranslocated into the cytosol and targeted for degradation by ubquitination (Ub) through the activity of E2 and E3 ligases such as SelL1. The H chain is deglycosylated by the N glyconase Pgn before proteolytic degradation. US2 appears to recruit a different set of proteins. Signal peptide peptidase is thought to work in concert with as yet defined protein(s) (?), which can lead to the enhanced proteasome mediated degradation of H chain.

homologue of yeast DER1p protein), VCP (p97)-interacting protein (VIMP) and an AAA-ATPase, p97,[46,47,48] which associates with the cofactors Np14 and Ufd1.[49] The Derlin-1 interaction is dependent on a glutamic acid at position 192 of the US11 TM domain[47] and appears to be important in the induction of the unfolded protein response, a cellular stress response to misfolded protein. In contrast, US2 does not induce the unfolded protein response,[50] coincident with it not using Derlin-1.[47] The only ERAD protein to be identified to date that associates with US2 is the signal peptide peptidase,[51] an ER-resident presenilin-type aspartic protease, which can cleave substrate polypeptides within TM regions (Fig. 2).[52,53]

The peptide loading complex – peptide loading and quality control

On association of β2m with MHC class I H chain, calnexin is displaced and replaced by a soluble ER lectin chaperone, calreticulin.[54] Like calnexin, calreticulin binds to monoglucosylated N-linked glycans and possesses an extended P domain which recruits ERp57.[55–57] Partially folded MHC class I molecules together with calreticulin associate with the transporter associated with antigen processing (TAP) via the MHC class I specific accessory molecule tapasin (TPN) to form the PLC.[54] The PLC allows the acquisition and optimization of the peptide cargo before transit to the cell surface (Fig. 3).[58,59] One important feature of the PLC is the co-operative binding nature of the respective components, which makes it difficult to study their individual effects.[1]

Tapasin, the central scaffold of the PLC?

It appears that the central scaffold for the PLC is TPN, which allows for stable TAP expression;[60] it is directly conjugated to ERp57 by a disulphide linkage between C95 of TPN and C57 of the **a** domain CXXC reactive motif of ERp57 (Fig. 3).[61] TPN, in concert with ERp57, can optimize the peptide cargo of PLC-associated MHC class I H chains.[62,63] The interaction with TPN maintains ERp57 within the PLC and inhibits the 'escape' pathway characteristic of oxidoreductases, i.e. prevents the oxidation–reduction cycle. This in effect maintains ERp57 in a predominantly reduced state within the PLC,[64,65] allowing the **a′** domain CXXC motif to interact with MHC class I H chains.[66] The C101–C164 bond is thought to be in a reduced state until optimal peptide is bound. One prediction of this model would be that MHC class I H chains should form a transient trimolecular complex with the ERp57–TPN conjugate.[14] Using alkylation to 'trap' transient disulphide intermediates, such a complex has recently been described,[66] which suggests that the ERp57–TPN conjugate controls the final redox state of the MHC class I H chain.[66]

The archetypal member of the PDI family, PDI, has recently been described as part of the PLC.[67] Using a series of elegant 'knockdown' mutants, PDI was ascribed a similar function to ERp57, in addition to binding peptides required for MHC class I loading. It is difficult to reconcile these findings with the function of the ERp57–TPN complex, but the detection of a putative complex between class I H chain–ERp57–TPN–PDI[66] suggests that

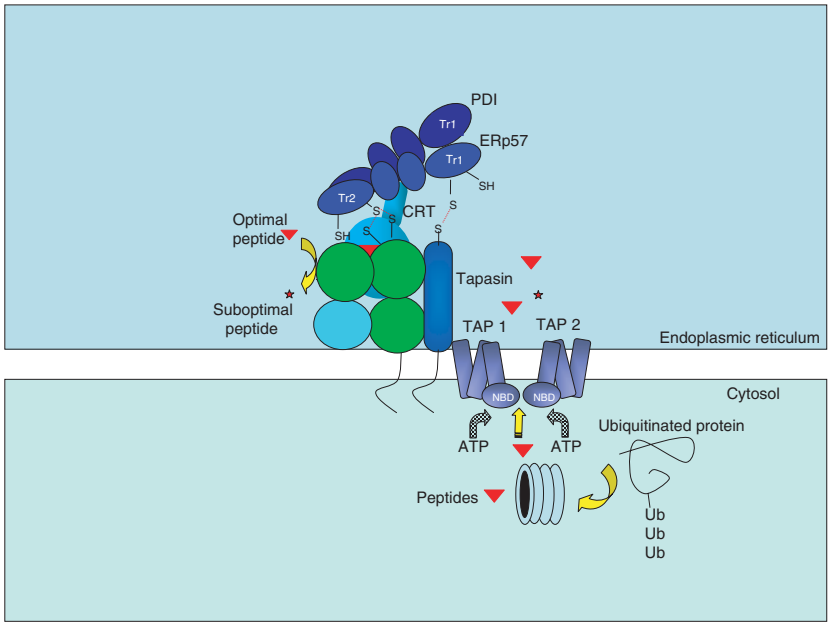

Figure 3. The peptide loading complex; partially folded major histocompatibility complex (MHC) class I molecules in association with calreticulin are incorporated into the peptide loading complex (PLC). MHC class I heavy (H) chains interact with tapasin (TPN) which tethers them to the transporter associated with antigen processing (TAP) complex. TPN is directly conjugated to ERp57 via a disulphide bond. The ERp57–TPN conjugate, possibly in concert with protein disulphide isomerise (PDI) allow for the acquisition of optimal peptides that are transported in an ATP-dependent manner by the TAP complex.

these molecules may work in concert. As ERp57 is in a reduced state, this limits its activity to reduction and isomerization. While not demonstrated within the PLC, other studies have shown that PDI predominantly participates in oxidation,[68,69] therefore it is possible that PDI and ERp57 work together to reduce and oxidize the C101–C164 bond. ERp57 knockout cells and knockdown experiments revealed that the redox status of MHC class I H chain remains unaffected,[70,71] probably reflecting the role of PDI in H chain oxidation.

Calreticulin plays a key role in allowing MHC class I molecules to acquire an optimal peptide cargo. Calreticulin knockout cells demonstrate poor MHC class I cell surface expression and a cargo with low-affinity peptides.[72] Interestingly, ERp57 knockout cells reveal poor recruitment of calreticulin to the PLC, which may account for the poor stability of cell surface MHC class I molecules, further illustrating the co-operative binding nature of the PLC.[70]

The PLC bottleneck – a prime target for subversion

The association with the PLC represents a potential bottleneck in MHC class I expression making it an effective focus for immune evasion (Fig. 4). There are broadly three types of PLC-mediated inhibition; (1) retention of MHC class I molecules within the ER and prevention of PLC interactions, (2) degradation of PLC components and (3) shutting off the crucial supply of peptide.

Retention of MHC class I molecules within the ER and prevention of PLC interactions

The earliest mechanism describing inhibition of MHC class I expression was the ER retention of MHC class I molecules by the 19 000 MW adenovirus gene product E19 using a KKXX ER-retention motif (Fig. 4a step 1).[73–75] Subsequently, E19 was demonstrated to increase the association of MHC class I molecules with the ubiquitous cellular amyloid precursor-like protein 2 (APLP2), which independently of E19, can reduce cell-surface-expressed MHC class I molecules.[76,77] The HCMV products US3 (Fig. 4a step 1) and US10, can either retain MHC class I molecules within the ER or delay their trafficking through the ER to the cell surface, respectively.[30,78–81] A product from the unique long (UL) region of HCMV, a tegument phosphoprotein pp71 encoded by UL82 and cowpox virus, can delay MHC class I trafficking in a hitherto undefined manner.[82,83] The mouse cytomegalovirus m152 protein retains H chains not in the ER, but within the ER–Golgi intermediate compartment.[84]

In addition to retention, E19 and US3 interfere with the action of TPN. E19 has been shown to bind TAP independently, preventing TPN-dependent alleles from entering the PLC, thereby inhibiting the activity of

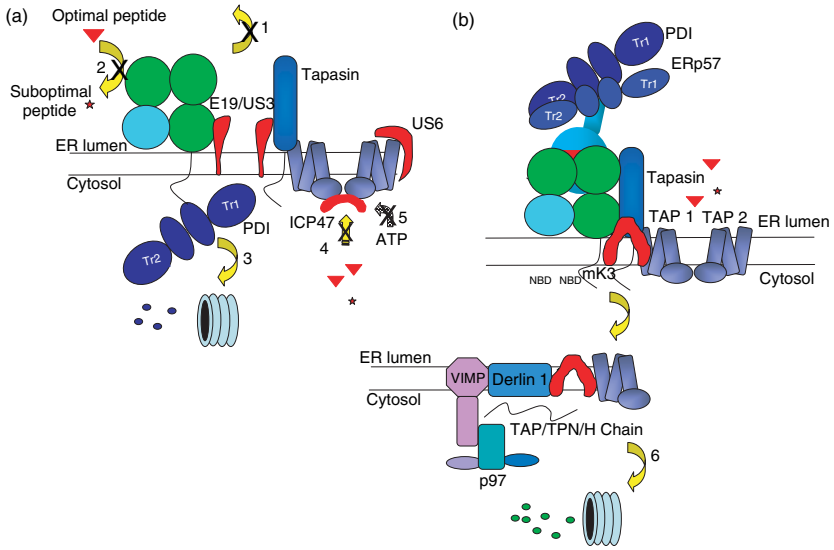

Figure 4. Inhibition of the peptide loading complex (PLC); the adenovirus E19 protein and the human cytomegalovirus (HCMV) US3 protein can both retain major histocompatibility complex (MHC) class I molecules, thereby preventing their transit to the cell surface (step 1). Both E19 and US3 can interfere with tapasin (TPN) activity (step 2), with US3 also enhancing the degradation of protein disulphide isomerise (PDI) in an undefined manner (step 3). The PLC is further targeted by inhibition of the peptide supply. ICP47 can act as a peptide inhibitor (step 4), while US6 prevents ATP hydrolysis (step 5). The mK3 protein inhibits the PLC by interacting with the MHC class I heavy (H) chain, transporter associated with antigen processing (TAP) and TPN, which can recruit Derlin1, VIMP and the p97 complex to enhance the degradation of these PLC components (step 6). Expression of MK3 is determined by TAP/TPN interactions, therefore modulating inhibition according to the level of antigen processing.

TPN.[85] US3 though can bind directly and independently to both TPN and TAP, inhibiting peptide optimization of PLC-associated MHC class I molecules (Fig. 4a step 2).[86]

Degradation of PLC components

TAP, TPN and MHC class I molecules within the PLC can be specifically selected for ERAD. The type III ER membrane protein mK3 (Fig. 4b) encoded by the murine γ-2 herpesvirus 68 is a member of the RING-CH family of viral immunoevasion genes that are found in different members of the γ2-herpesvirus and in the unrelated poxviruses.[87] Unusually they employ the same mechanism of action, i.e. ubiquitination, to target immunoreceptors for down-regulation. These proteins share an N-terminal zinc binding motif with homology to RING fingers and plant homeobox domain motifs, which are found in a family of proteins that possess Ub-E3 ligase activity.[88–90]

The mK3 directly associates with MHC class I molecules, probably through CYT domain interactions,[88] leading to the ubiquitination of membrane-bound and glycosylated MHC class I molecules.[91,92] Ubiquitination is dependent on the RING domain and N terminus of the mK3 protein[88,91] and leads to the dislocation and targeted degradation of MHC class I molecules within the PLC by exploiting similar components to those used by US11, such as p97 (Fig. 4b step 6).[46,47]

The non-classical glycosylphosphatidylinositol membrane-anchored MHC class I molecule Qa-2 (which lacks a cytoplasmic domain to enable ubiquitination) is also subject to mK3-mediated ERAD, but via mK3's ability to enhance the degradation of TPN and TAP, especially TAP2. This is especially intriguing because TAP2 was demonstrated to possess a docking site for TPN, allowing effective PLC formation.[93] The mK3 protein requires intact TPN to degrade class I H chains and only the TM/CYT domain of TPN to degrade TAP.[94] Expression and function of mK3 are therefore coupled to the level of antigen processing and presentation via TAP/TPN interactions, which may explain why mK3 MHC class I degradation is resistant to interferon-γ-induced up-regulation.[95] UL49.5, a 9000 MW protein encoded by varicelloviruses such as bovine herpesvirus 1 and pseudorabies virus also inactivate TAP by promoting its degradation as well as inducing conformational changes that inhibit peptide translocation.[96,97] US3 appears to target PDI for degradation (Fig. 4a step 3), hence interfering with the redox status of MHC class I molecules.[67] This would appear to be an unusual target for degradation because PDI can be involved in viral protein folding. It remains to be determined whether it is the PLC-associated PDI that is specifically targeted in preference to the general cellular pool.

Shutting off the crucial supply of peptide

Inhibiting peptide supply by interfering with TAP function is an effective way of down-regulating MHC class I

expression. The herpes simplex virus ICP47 protein and the HCMV US6 protein are the best-characterized TAP inhibitors (Fig. 4a). ICP47 is an 88-amino-acid protein that occupies the peptide binding site located in the cytosol and acts as a high-affinity peptide competitor for peptide binding (Fig. 4a step 4).[98–102] US6, a 21 000 MW ER luminal protein, prevents binding of ATP especially by TAP1, even though the nucleotide binding domain sites are cytosolically located (Fig. 4a step 5). Binding to the ER luminal side of TAP, US6 appears to exert or maintain a specific conformational change inhibiting ATP hydrolysis and thereby preventing peptide translocation.[103] ICP47 has a differential affect on HLA alleles depending on their requirement for the PLC. This was demonstrated for two subtypes of HLA-B44, i.e. 02 and 05, which differ by a single aspartic acid to tyrosine change at position 116, respectively. HLA-B4405, which is TPN independent[58] and therefore does not require the PLC for expression, was found to be more resistant to TAP inhibition. Such an observation raises the possibility that certain MHC polymorphisms have been selected to overcome pathogen effects on their biosynthesis.[104]

Transit to the cell surface

The transit of MHC class I molecules from the ER to the cell surface, does not only depend on optimal peptide binding. Conformational changes within the TAP complex can govern MHC class I release from the PLC. TAP nucleotide binding domain mutants and nucleotide depletion experiments suggest that peptide-mediated dissociation of MHC class I molecules depends on conformational signals arising from TAP.[105,106] Upon dissociation from TAP, peptide-loaded class I molecules can cluster at ER exit sites, and associate with a putative transport receptor BAP31, a 28 000 MW TM protein previously found associated with immunoglobulin D.[107] These exit sites exclude TAP and TPN, which strongly suggests that MHC class I ER export is highly regulated and receptor mediated.

Down-regulation of cell surface molecules

Though fully folded and peptide loaded, MHC class I molecules continue to remain susceptible targets and are primarily subjected to two general modes of inhibition, either mis-sorting or enhanced internalization. The best-described inhibitors of MHC class I cell surface molecules are those encoded by Kaposi's sarcoma-associated herpesvirus (KSHV) and the human immunodeficiency virus (HIV).

Two proteins encoded by KSHV, K3 and K5, target MHC class I molecules at the cell surface. K3 and K5 are members of the same family of viral proteins as mK3, which share an N-terminal RING-CH domain and act as E3 Ub-ligases.[108] While mK3 appears to target ER-resident MHC class I molecules, K3 and K5 target fully mature cell-surface-expressed molecules for ubiquitination, which enhances internalization and trafficking to endosomal/lysosomal compartments.[90] K3 can down-regulate both classical and non-classical class I molecules whereas K5 only targets HLA-A and HLA-B alleles with substrate specificity governed by TM interactions.[109,110] K3 and K5 differ in their use of target K residues, reflecting the possible use of different E2 Ub-conjugating enzymes.[108] Unusually, K3 can also ubiquitinate cysteine residues,[111] which form a labile thiol–ester bond, whereas K5 is highly dependent on cytoplasmic K residues.

A conserved area of 41 amino acids within the C-terminal domain containing several putative sorting motifs, distal to the second TM domain of K3, appears to be critical for targeting MHC class I H chains to lysosomes.[112] E2 Ub-conjugating enzymes, in particular UbcH5b/c, are involved in monoubiquitinating MHC class I H chains, which in turn initiate polyubiquitination by Ubc13. Polyubiquitination via K-63 Ub chains (Ub molecules linked via K at position 63 of Ub) of MHC class I H chains, leads to clathrin-dependent internalization, which requires the epsin1 endocytic adaptor (a clathrin adaptor implicated in the recognition of ubiquitinated plasma membrane proteins through their Ub-interacting motifs).[113] Endocytosed MHC class I molecules traffic via multivesicular bodies to lysosomes, using the endosomal sorting complex 1 machinery, of which TSG101 binds Ub and sorts ubiquitinated proteins to multivesicular bodies.[90]

The HIV Nef protein uses the endocytic sorting machinery to misdirect MHC class I molecules away from the cell surface.[114–116] Nef is a 27 000 MW myristolated protein that associates with the CYT domain of HLA-A and HLA-B molecules[116] and uses a clathrin-independent mechanism to sequester MHC class I to the trans-Golgi network (TGN). Nef seems to co-opt the internalization and recycling pathway of MHC class I molecules, which is regulated by the small GTPase, ADP-ribosylation factor 6.[115] Nef contains three motifs governing MHC internalization; ^{62}EEEE^{65} and ^{72}PXXP^{75} are required for internalization, while methionine^{20} within an amphiphatic α-helix is necessary for TGN sequestration. The ^{62}EEEE^{65} associates with phosphofurin acidic cluster sorting protein-1, which sorts Nef to the TGN, but does not facilitate MHC class I sequestration. By localizing to the TGN, Nef activates the internalization of MHC class I molecules in a ^{72}PXXP^{75} and possibly phosphatidylinositide 3-kinase-dependent manner. Nef activity is different depending on the cell type.[117] In T cells, Nef disrupts MHC class I trafficking to the cell surface, using an adaptor protein 1-dependent pathway to redirect the molecules to lysosomes.[117–119] Adaptor protein 1 is a heterotetrameric protein complex, mediating vesicular transport between the TGN and endolysosomal pathways, by linking cargo

proteins to clathrin.[119] Nef can target MHC class I molecules early in the folding pathway when expressed in T cells, by preferentially binding newly synthesized hypophosphorylated H chains. The phosphorylation status of Nef interacting MHC class I molecules may in part be responsible for the more pronounced effects on slow-maturing MHC class I molecules, leading to their accumulation within the TGN and diversion to lysosomes.[117]

Cross and criss-cross presentation – evolution to circumvent potential biosynthetic inhibition?

Apart from the sequence polymorphism, MHC class I molecules have also evolved alternative pathways for accessing pathogen-derived peptides and potentially overcoming the plethora of inhibitory mediators. MHC class I molecules can present peptide from exogenously derived antigen, via a process termed 'cross-presentation' of which there appear to be several defined pathways.[120] Cross-presentation enables MHC class I–peptide presentation of pathogen-derived antigens prior to them becoming established and arming their immunoevasion strategies. Of particular note, the production of interferon-α/β, which is produced during viral infection,[121] was found to also stimulate cross-presentation independent of $CD4^+$ T-cell help and CD40–CD40 ligand interactions.[122] A recently described 'criss-cross' pathway of presentation whereby peptide from one cell can cross over through gap junctions and be presented by neighbouring cells[123] would not only limit the spread of infection, but would allow pathogen-derived peptides to be presented from an infected cell that may have had its MHC machinery disarmed.

Pathogens such as HCMV and herpes simplex virus, though armed to provide an effective means of overcoming cytotoxic T-cell recognition can trigger strong T-cell responses. Such infections become a problem to the host during times of immunosuppression. It is possible that cross-presentation could be the answer to the apparent paradox of host immunity in the face of such immune evasion strategies.[124]

Although this review has concentrated on the MHC class I biosynthetic pathway and the ability of viral pathogens to down-modulate or inhibit this pathway, it remains to be seen whether bacteria and other pathogens such as helminths can also target the biosynthesis of MHC class I molecules. Bacterial evasion of the MHC has been described for class II molecules[125] and bacteria such *Chlamydia trachomatis* and *Mycobacterium tuberculosis* can inhibit classical and non-classical class I molecules at the transcriptional level.[125–127] Furthermore, certain bacteria such as *Salmonella enterica* can affect antigen-processing pathways[128] but appear not to undergo clearance unless expressed with particular MHC proteins,[129] raising the possibility that other non-antigen-presenting functions of MHC molecules may be co-opted by pathogens for their own survival.

References

1 Antoniou AN, Powis SJ, Elliott T. Assembly and export of MHC class I peptide ligands. *Curr Opin Immunol* 2003; **15**:75–81.

2 Degen E, Cohen-Doyle MF, Williams DB. Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both beta 2-microglobulin and peptide. *J Exp Med* 1992; **175**:1653–61.

3 Zhang Q, Tector M, Salter RD. Calnexin recognizes carbohydrate and protein determinants of class I major histocompatibility complex molecules. *J Biol Chem* 1995; **270**:3944–8.

4 Zhang Q, Salter RD. Distinct patterns of folding and interactions with calnexin and calreticulin in human class I MHC proteins with altered N-glycosylation. *J Immunol* 1998; **160**:831–7.

5 Ellgaard L, Helenius A. ER quality control: towards an understanding at the molecular level. *Curr Opin Cell Biol* 2001; **13**:431–7.

6 Hammond C, Braakman I, Helenius A. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. *Proc Natl Acad Sci U S A* 1994; **91**:913–7.

7 Schrag JD, Bergeron JJ, Li Y, Borisova S, Hahn M, Thomas DY, Cygler M. The structure of calnexin, an ER chaperone involved in quality control of protein folding. *Mol Cell* 2001; **8**:633–44.

8 Wilson CM, Farmery MR, Bulleid NJ. Pivotal role of calnexin and mannose trimming in regulating the endoplasmic reticulum-associated degradation of major histocompatibility complex class I heavy chain. *J Biol Chem* 2000; **275**:21224–32.

9 Sadasivan BK, Cariappa A, Waneck GL, Cresswell P. Assembly, peptide loading, and transport of MHC class I molecules in a calnexin-negative cell line. *Cold Spring Harb Symp Quant Biol* 1995; **60**:267–75.

10 Walker KW, Gilbert HF. Scanning and escape during protein-disulfide isomerase-assisted protein folding. *J Biol Chem* 1997; **272**:8845–8.

11 Ellgaard L, Frickel EM. Calnexin, calreticulin, and ERp57: teammates in glycoprotein folding. *Cell Biochem Biophys* 2003; **39**:223–47.

12 Beynon-Jones SM, Antoniou AN, Powis SJ. Mutational analysis of the oxidoreductase ERp57 reveals the importance of the two central residues in the redox motif. *FEBS Lett* 2006; **580**:1897–902.

13 Antoniou AN, Santos SG, Campbell EC, Lynch S, Arosa FA, Powis SJ. ERp57 interacts with conserved cysteine residues in the MHC class I peptide-binding groove. *FEBS Lett* 2007; **581**:1988–92.

14 Antoniou AN, Ford S, Alphey M, Osborne A, Elliott T, Powis SJ. The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules. *EMBO J* 2002; **21**:2655–63.

15 Tortorella D, Story CM, Huppa JB *et al.* Dislocation of type I membrane proteins from the ER to the cytosol is sensitive to changes in redox potential [published erratum appears in J Cell Biol 1999 May 3; 145(3):following 642]. *J Cell Biol* 1998; **142**:365–76.

16 Nossner E, Parham P. Species-specific differences in chaperone interaction of human and mouse major histocompatibility complex class I molecules. *J Exp Med* 1995; **181**:327–37.
17 Antoniou AN, Ford S, Taurog JD, Butcher GW, Powis SJ. Formation of HLA-B27 homodimers and their relationship to assembly kinetics. *J Biol Chem* 2004; **279**:8895–902.
18 Hellman R, Vanhove M, Lejeune A, Stevens FJ, Hendershot LM. The *in vivo* association of BiP with newly synthesized proteins is dependent on the rate and stability of folding and not simply on the presence of sequences that can bind to BiP. *J Cell Biol* 1999; **144**:21–30.
19 Hendershot LM. The ER function BiP is a master regulator of ER function. *Mt Sinai J Med* 2004; **71**:289–97.
20 Tran TM, Satumtira N, Dorris ML, May E, Wang A, Furuta E, Taurog JD. HLA-B27 in transgenic rats forms disulfide-linked heavy chain oligomers and multimers that bind to the chaperone BiP. *J Immunol* 2004; **172**:5110–9.
21 Molinari M, Helenius A. Chaperone selection during glycoprotein translocation into the endoplasmic reticulum. *Science* 2000; **288**:331–3.
22 Molinari M, Helenius A. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. *Nature* 1999; **402**:90–3.
23 Raposo G, van Santen HM, Leijendekker R, Geuze HJ, Ploegh HL. Misfolded major histocompatibility complex class I molecules accumulate in an expanded ER-Golgi intermediate compartment. *J Cell Biol* 1995; **131**:1403–19.
24 Hughes EA, Hammond C, Cresswell P. Misfolded major histocompatibility complex class I heavy chains are translocated into the cytoplasm and degraded by the proteasome. *Proc Natl Acad Sci U S A* 1997; **94**:1896–901.
25 Wiertz EJ, Jones TR, Sun L, Bogyo M, Geuze HJ, Ploegh HL. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* 1996; **84**:769–79.
26 Spiliotis ET, Pentcheva T, Edidin M. Probing for membrane domains in the endoplasmic reticulum: retention and degradation of unassembled MHC class I molecules. *Mol Biol Cell* 2002; **13**:1566–81.
27 Kamhi-Nesher S, Shenkman M, Tolchinsky S, Fromm SV, Ehrlich R, Lederkremer GZ. A novel quality control compartment derived from the endoplasmic reticulum. *Mol Biol Cell* 2001; **12**:1711–23.
28 Ciechanover A, Ben-Saadon R. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol* 2004; **14**:103–6.
29 Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998; **67**:425–79.
30 Ahn K, Angulo A, Ghazal P, Peterson PA, Yang Y, Fruh K. Human cytomegalovirus inhibits antigen presentation by a sequential multistep process. *Proc Natl Acad Sci U S A* 1996; **93**:10990–5.
31 Barel MT, Ressing M, Pizzato N, van Leeuwen D, Le Bouteiller P, Lenfant F, Wiertz EJ. Human cytomegalovirus-encoded US2 differentially affects surface expression of MHC class I locus products and targets membrane-bound, but not soluble HLA-G1 for degradation. *J Immunol* 2003; **171**:6757–65.
32 Furman MH, Ploegh HL, Tortorella D. Membrane-specific, host-derived factors are required for US2- and US11-mediated degradation of major histocompatibility complex class I molecules. *J Biol Chem* 2002; **277**:3258–67.
33 Barel MT, Pizzato N, van Leeuwen D, Bouteiller PL, Wiertz EJ, Lenfant F. Amino acid composition of alpha1/alpha2 domains and cytoplasmic tail of MHC class I molecules determine their susceptibility to human cytomegalovirus US11-mediated down-regulation. *Eur J Immunol* 2003; **33**:1707–16.
34 Gewurz BE, Gaudet R, Tortorella D, Wang EW, Ploegh HL, Wiley DC. Antigen presentation subverted: structure of the human cytomegalovirus protein US2 bound to the class I molecule HLA-A2. *Proc Natl Acad Sci U S A* 2001; **98**:6794–9.
35 Gewurz BE, Wang EW, Tortorella D, Schust DJ, Ploegh HL. Human cytomegalovirus US2 endoplasmic reticulum-lumenal domain dictates association with major histocompatibility complex class I in a locus-specific manner. *J Virol* 2001; **75**:5197–204.
36 Blom D, Hirsch C, Stern P, Tortorella D, Ploegh HL. A glycosylated type I membrane protein becomes cytosolic when peptide: N-glycanase is compromised. *EMBO J* 2004; **23**:650–8.
37 Barel MT, Hassink GC, van Voorden S, Wiertz EJ. Human cytomegalovirus-encoded US2 and US11 target unassembled MHC class I heavy chains for degradation. *Mol Immunol* 2006; **43**:1258–66.
38 Shamu CE, Story CM, Rapoport TA, Ploegh HL. The pathway of US11-dependent degradation of MHC class I heavy chains involves a ubiquitin-conjugated intermediate. *J Cell Biol* 1999; **147**:45–58.
39 Hirsch C, Blom D, Ploegh HL. A role for N-glycanase in the cytosolic turnover of glycoproteins. *EMBO J* 2003; **22**:1036–46.
40 Rehm A, Engelsberg A, Tortorella D, Korner IJ, Lehmann I, Ploegh HL, Hopken UE. Human cytomegalovirus gene products US2 and US11 differ in their ability to attack major histocompatibility class I heavy chains in dendritic cells. *J Virol* 2002; **76**:5043–50.
41 Furman MH, Loureiro J, Ploegh HL, Tortorella D. Ubiquitinylation of the cytosolic domain of a type I membrane protein is not required to initiate its dislocation from the endoplasmic reticulum. *J Biol Chem* 2003; **278**:34804–11.
42 Ribaudo RK, Margulies DH. Independent and synergistic effects of disulfide bond formation, beta 2-microglobulin, and peptides on class I MHC folding and assembly in an *in vitro* translation system. *J Immunol* 1992; **149**:2935–44.
43 Rajagopalan S, Brenner MB. Calnexin retains unassembled major histocompatibility complex class I free heavy chains in the endoplasmic reticulum. *J Exp Med* 1994; **180**:407–12.
44 Jackson MR, Cohen-Doyle MF, Peterson PA, Williams DB. Regulation of MHC class I transport by the molecular chaperone, calnexin (p88, IP90). *Science* 1994; **263**:384–7.
45 Mueller B, Lilley BN, Ploegh HL. SEL1L, the homologue of yeast Hrd3p, is involved in protein dislocation from the mammalian ER. *J Cell Biol* 2006; **175**:261–70.
46 Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004; **429**:841–7.
47 Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 2004; **429**:834–40.
48 Kothe M, Ye Y, Wagner JS, De Luca HE, Kern E, Rapoport TA, Lencer WI. Role of p97 AAA-ATPase in the retrotranslocation of the cholera toxin A1 chain, a non-ubiquitinated substrate. *J Biol Chem* 2005; **280**:28127–32.

49 Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 2001; **414**:652–6.

50 Tirosh B, Iwakoshi NN, Lilley BN, Lee AH, Glimcher LH, Ploegh HL. Human cytomegalovirus protein US11 provokes an unfolded protein response that may facilitate the degradation of class I major histocompatibility complex products. *J Virol* 2005; **79**:2768–79.

51 Loureiro J, Lilley BN, Spooner E, Noriega V, Tortorella D, Ploegh HL. Signal peptide peptidase is required for dislocation from the endoplasmic reticulum. *Nature* 2006; **441**:894–7.

52 Friedmann E, Lemberg MK, Weihofen A, Dev KK, Dengler U, Rovelli G, Martoglio B. Consensus analysis of signal peptide peptidase and homologous human aspartic proteases reveals opposite topology of catalytic domains compared with presenilins. *J Biol Chem* 2004; **279**:50790–8.

53 Weihofen A, Binns K, Lemberg MK, Ashman K, Martoglio B. Identification of signal peptide peptidase, a presenilin-type aspartic protease. *Science* 2002; **296**:2215–8.

54 Sadasivan B, Lehner PJ, Ortmann B, Spies T, Cresswell P. Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP. *Immunity* 1996; **5**:103–14.

55 Frickel EM, Riek R, Jelesarov I, Helenius A, Wuthrich K, Ellgaard L. TROSY-NMR reveals interaction between ERp57 and the tip of the calreticulin P-domain. *Proc Natl Acad Sci U S A* 2002; **99**:1954–9.

56 Elliott JG, Oliver JD, High S. The thiol-dependent reductase ERp57 interacts specifically with N-glycosylated integral membrane proteins. *J Biol Chem* 1997; **272**:13849–55.

57 Oliver JD, Roderick HL, Llewellyn DH, High S. ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin. *Mol Biol Cell* 1999; **10**:2573–82.

58 Williams AP, Peh CA, Purcell AW, McCluskey J, Elliott T. Optimization of the MHC class I peptide cargo is dependent on tapasin. *Immunity* 2002; **16**:509–20.

59 Chen M, Bouvier M. Analysis of interactions in a tapasin/class I complex provides a mechanism for peptide selection. *EMBO J* 2007; **26**:1681–90.

60 Lehner PJ, Surman MJ, Cresswell P. Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line .220. *Immunity* 1998; **8**:221–31.

61 Dick TP, Bangia N, Peaper DR, Cresswell P. Disulfide bond isomerization and the assembly of MHC class I–peptide complexes. *Immunity* 2002; **16**:87–98.

62 Wearsch PA, Cresswell P. Selective loading of high-affinity peptides onto major histocompatibility complex class I molecules by the tapasin-ERp57 heterodimer. *Nat Immunol* 2007; **8**:873–81.

63 Kienast A, Preuss M, Winkler M, Dick TP. Redox regulation of peptide receptivity of major histocompatibility complex class I molecules by ERp57 and tapasin. *Nat Immunol* 2007; **8**:864–72.

64 Antoniou AN, Powis SJ. Characterization of the ERp57–Tapasin complex by rapid cellular acidification and thiol modification. *Antioxid Redox Signal* 2003; **5**:375–9.

65 Peaper DR, Wearsch PA, Cresswell P. Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. *EMBO J* 2005; **24**:3613–23.

66 Santos SG, Campbell EC, Lynch S, Wong V, Antoniou AN, Powis SJ. MHC class I–ERp57–tapasin interactions within the peptide-loading complex. *J Biol Chem* 2007; **282**:17587–93.

67 Park B, Lee S, Kim E, Cho K, Riddell SR, Cho S, Ahn K. Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. *Cell* 2006; **127**:369–82.

68 Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* 2004; **164**:341–6.

69 Tu BP, Ho-Schleyer SC, Travers KJ, Weissman JS. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* 2000; **290**:1571–4.

70 Garbi N, Tanaka S, Momburg F, Hammerling GJ. Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. *Nat Immunol* 2006; **7**:93–102.

71 Zhang Y, Baig E, Williams DB. Functions of ERp57 in the folding and assembly of major histocompatibility complex class I molecules. *J Biol Chem* 2006; **281**:14622–31.

72 Gao B, Adhikari R, Howarth M *et al.* Assembly and antigen-presenting function of MHC class I molecules in cells lacking the ER chaperone calreticulin. *Immunity* 2002; **16**:99–109.

73 Andersson M, Paabo S, Nilsson T, Peterson PA. Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. *Cell* 1985; **43**:215–22.

74 Cox JH, Bennink JR, Yewdell JW. Retention of adenovirus E19 glycoprotein in the endoplasmic reticulum is essential to its ability to block antigen presentation. *J Exp Med* 1991; **174**:1629–37.

75 Burgert HG, Kvist S. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell* 1985; **41**:987–97.

76 Sester M, Feuerbach D, Frank R, Preckel T, Gutermann A, Burgert HG. The amyloid precursor-like protein 2 associates with the major histocompatibility complex class I molecule K(d). *J Biol Chem* 2000; **275**:3645–54.

77 Morris CR, Petersen JL, Vargas SE *et al.* The amyloid precursor-like protein 2 and the adenoviral E3/19K protein both bind to a conformational site on H-2Kd and regulate H-2Kd expression. *J Biol Chem* 2003; **278**:12618–23.

78 Furman MH, Dey N, Tortorella D, Ploegh HL. The human cytomegalovirus US10 gene product delays trafficking of major histocompatibility complex class I molecules. *J Virol* 2002; **76**:11753–6.

79 Jones TR, Wiertz EJ, Sun L, Fish KN, Nelson JA, Ploegh HL. Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proc Natl Acad Sci U S A* 1996; **93**:11327–33.

80 Lee S, Yoon J, Park B *et al.* Structural and functional dissection of human cytomegalovirus US3 in binding major histocompatibility complex class I molecules. *J Virol* 2000; **74**:11262–9.

81 Gruhler A, Peterson PA, Fruh K. Human cytomegalovirus immediate early glycoprotein US3 retains MHC class I molecules by transient association. *Traffic* 2000; **1**:318–25.

82 Trgovcich J, Cebulla C, Zimmerman P, Sedmak DD. Human cytomegalovirus protein pp71 disrupts major histocompatibility complex class I cell surface expression. *J Virol* 2006; **80**:951–63.

83 Dasgupta A, Hammarlund E, Slifka MK, Fruh K. Cowpox virus evades CTL recognition and inhibits the intracellular transport of MHC class I molecules. *J Immunol* 2007; **178**:1654–61.

84 Ziegler H, Thale R, Lucin P *et al.* A mouse cytomegalovirus glycoprotein retains MHC class I complexes in the ERGIC/cis-Golgi compartments. *Immunity* 1997; **6**:57–66.

85 Bennett EM, Bennink JR, Yewdell JW, Brodsky FM. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. *J Immunol* 1999; **162**:5049–52.

86 Park B, Kim Y, Shin J, Lee S, Cho K, Fruh K, Ahn K. Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. *Immunity* 2004; **20**:71–85.

87 Virgin HWT, Latreille P, Wamsley P, Hallsworth K, Weck KE, Dal Canto AJ, Speck SH. Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J Virol* 1997; **71**:5894–904.

88 Boname JM, Stevenson PG. MHC class I ubiquitination by a viral PHD/LAP finger protein. *Immunity* 2001; **15**:627–36.

89 Coscoy L, Sanchez DJ, Ganem D. A novel class of herpesvirus-encoded membrane-bound E3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition. *J Cell Biol* 2001; **155**:1265–73.

90 Hewitt EW, Duncan L, Mufti D, Baker J, Stevenson PG, Lehner PJ. Ubiquitylation of MHC class I by the K3 viral protein signals internalization and TSG101-dependent degradation. *EMBO J* 2002; **21**:2418–29.

91 Wang X, Connors R, Harris MR, Hansen TH, Lybarger L. Requirements for the selective degradation of endoplasmic reticulum-resident major histocompatibility complex class I proteins by the viral immune evasion molecule mK3. *J Virol* 2005; **79**:4099–108.

92 Lybarger L, Wang X, Harris MR, Virgin HWt, Hansen TH. Virus subversion of the MHC class I peptide-loading complex. *Immunity* 2003; **18**:121–30.

93 Leonhardt RM, Keusekotten K, Bekpen C, Knittler MR. Critical role for the tapasin-docking site of TAP2 in the functional integrity of the MHC class I–peptide-loading complex. *J Immunol* 2005; **175**:5104–14.

94 Boname JM, May JS, Stevenson PG. The murine gamma-herpesvirus-68 MK3 protein causes TAP degradation independent of MHC class I heavy chain degradation. *Eur J Immunol* 2005; **35**:171–9.

95 Boname JM, de Lima BD, Lehner PJ, Stevenson PG. Viral degradation of the MHC class I peptide loading complex. *Immunity* 2004; **20**:305–17.

96 Koppers-Lalic D, Reits EA, Ressing ME *et al.* Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing. *Proc Natl Acad Sci U S A* 2005; **102**:5144–9.

97 van Hall T, Laban S, Koppers-Lalic D, Koch J, Precup C, Asmawidjaja P, Offringa R, Wiertz EJ. The varicellovirus-encoded TAP inhibitor UL49.5 regulates the presentation of CTL epitopes by Qa-1b1. *J Immunol* 2007; **178**:657–62.

98 Hill A, Jugovic P, York I, Russ G, Bennink J, Yewdell J, Ploegh H, Johnson D. Herpes simplex virus turns off the TAP to evade host immunity. *Nature* 1995; **375**:411–5.

99 Fruh K, Ahn K, Djaballah H, Sempe P, van Endert PM, Tampe R, Peterson PA, Yang Y. A viral inhibitor of peptide transporters for antigen presentation. *Nature* 1995; **375**:415–8.

100 Ahn K, Meyer TH, Uebel S *et al.* Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus ICP47. *EMBO J* 1996; **15**:3247–55.

101 Tomazin R, Hill AB, Jugovic P, York I, van Endert P, Ploegh HL, Andrews DW, Johnson DC. Stable binding of the herpes simplex virus ICP47 protein to the peptide binding site of TAP. *EMBO J* 1996; **15**:3256–66.

102 Galocha B, Hill A, Barnett BC *et al.* The active site of ICP47, a herpes simplex virus-encoded inhibitor of the major histocompatibility complex (MHC)-encoded peptide transporter associated with antigen processing (TAP), maps to the NH_2-terminal 35 residues. *J Exp Med* 1997; **185**:1565–72.

103 Hewitt EW, Gupta SS, Lehner PJ. The human cytomegalovirus gene product US6 inhibits ATP binding by TAP. *EMBO J* 2001; **20**:387–96.

104 Zernich D, Purcell AW, Macdonald WA *et al.* Natural HLA class I polymorphism controls the pathway of antigen presentation and susceptibility to viral evasion. *J Exp Med* 2004; **200**:13–24.

105 Antoniou AN, Ford S, Pilley ES, Blake N, Powis SJ. Interactions formed by individually expressed TAP1 and TAP2 polypeptide subunits. *Immunology* 2002; **106**:182–9.

106 Knittler MR, Alberts P, Deverson EV, Howard JC. Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules. *Curr Biol* 1999; **9**:999–1008.

107 Adachi T, Schamel WW, Kim KM, Watanabe T, Becker B, Nielsen PJ, Reth M. The specificity of association of the IgD molecule with the accessory proteins BAP31/BAP29 lies in the IgD transmembrane sequence. *EMBO J* 1996; **15**:1534–41.

108 Lehner PJ, Hoer S, Dodd R, Duncan LM. Downregulation of cell surface receptors by the K3 family of viral and cellular ubiquitin E3 ligases. *Immunol Rev* 2005; **207**:112–25.

109 Sanchez DJ, Coscoy L, Ganem D. Functional organization of MIR2, a novel viral regulator of selective endocytosis. *J Biol Chem* 2002; **277**:6124–30.

110 Wang X, Lybarger L, Connors R, Harris MR, Hansen TH. Model for the interaction of gammaherpesvirus 68 RING-CH finger protein mK3 with major histocompatibility complex class I and the peptide-loading complex. *J Virol* 2004; **78**:8673–86.

111 Cadwell K, Coscoy L. Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase. *Science* 2005; **309**:127–30.

112 Means RE, Ishido S, Alvarez X, Jung JU. Multiple endocytic trafficking pathways of MHC class I molecules induced by a Herpesvirus protein. *EMBO J* 2002; **21**:1638–49.

113 Hofmann K, Falquet L. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem Sci* 2001; **26**:347–50.

114 Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nat Med* 1996; **2**:338–42.

115 Blagoveshchenskaya AD, Thomas L, Feliciangeli SF, Hung CH, Thomas G. HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. *Cell* 2002; **111**:853–66.

116 Williams M, Roeth JF, Kasper MR, Fleis RI, Przybycin CG, Collins KL. Direct binding of human immunodeficiency virus type 1 Nef to the major histocompatibility complex class I (MHC-I) cytoplasmic tail disrupts MHC-I trafficking. *J Virol* 2002; **76**:12173–84.

117 Kasper MR, Roeth JF, Williams M, Filzen TM, Fleis RI, Collins KL. HIV-1 Nef disrupts antigen presentation early in the secretory pathway. *J Biol Chem* 2005; **280**:12840–8.

118 Kasper MR, Collins KL. Nef-mediated disruption of HLA-A2 transport to the cell surface in T cells. *J Virol* 2003; **77**:3041–9.

119 Roeth JF, Williams M, Kasper MR, Filzen TM, Collins KL. HIV-1 Nef disrupts MHC-I trafficking by recruiting AP-1 to the MHC-I cytoplasmic tail. *J Cell Biol* 2004; **167**:903–13.

120 Rock KL, Shen L. Cross-presentation: underlying mechanisms and role in immune surveillance. *Immunol Rev* 2005; **207**:166–83.

121 Goodbourn S, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J Gen Virol* 2000; **81**:2341–64.

122 Le Bon A, Etchart N, Rossmann C, Ashton M, Hou S, Gewert D, Borrow P, Tough DF. Cross-priming of $CD8^+$ T cells stimulated by virus-induced type I interferon. *Nat Immunol* 2003; **4**:1009–15.

123 Neijssen J, Herberts C, Drijfhout JW, Reits E, Janssen L, Neefjes J. Cross-presentation by intercellular peptide transfer through gap junctions. *Nature* 2005; **434**:83–8.

124 Pollara G, Katz DR, Chain BM. The host response to herpes simplex virus infection. *Curr Opin Infect Dis* 2004; **17**:199–203.

125 Hornef MW, Wick MJ, Rhen M, Normark S. Bacterial strategies for overcoming host innate and adaptive immune responses. *Nat Immunol* 2002; **3**:1033–40.

126 Stenger S, Niazi KR, Modlin RL. Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis*. *J Immunol* 1998; **161**:3582–8.

127 Zhong G, Liu L, Fan T, Fan P, Ji H. Degradation of transcription factor RFX5 during the inhibition of both constitutive and interferon gamma-inducible major histocompatibility complex class I expression in chlamydia-infected cells. *J Exp Med* 2000; **191**:1525–34.

128 Cheminay C, Mohlenbrink A, Hensel M. Intracellular *Salmonella* inhibit antigen presentation by dendritic cells. *J Immunol* 2005; **174**:2892–9.

129 Laitio P, Virtala M, Salmi M, Pelliniemi LJ, Yu DT, Granfors K. HLA-B27 modulates intracellular survival of *Salmonella enteritidis* in human monocytic cells. *Eur J Immunol* 1997; **27**:1331–8.